

# Gut bacterial and clinical biomarkers characterization in obese patient before and after bariatric surgery

Does bariatric surgery make patients' lives better?

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## Introduction

In the past 40 years, the rapid economic growth, globalization, and urbanization have led to a sharp rise in overweight and obesity rates in China. Overweight can have a high risk in causing cardiovascular disease, diabetes and even cancers. Bariatric surveys, such as sleeve gastrectomy, gastric bypass and adjustable gastric banding, are proven to not only have benefits in weight losing, but also significant improvement in life quality.

The normal human gut microbiota predominantly consists of Firmicutes, Bacteroides, Proteus, Actinomycetes, Fusobacteria, and Verrucomicrobia, with Firmicutes and Bacteroides being the most abundant. At the genus level, Lachnospiraceae, Ruminococcaceae, Acidaminococcaceae, and Coriobacteriaceae are more predominant in the postoperative gut microbiota. Numerous studies have suggested a link between obesity and gut bacteria, with disruptions in bacterial composition associated with insulin resistance, metabolic disorders, and chronic inflammation [1][2].

This study examines changes in gut bacteria and clinical biomarkers six years before and after bariatric surgery, aiming to assess the impact of the surgery on patients' quality of life.

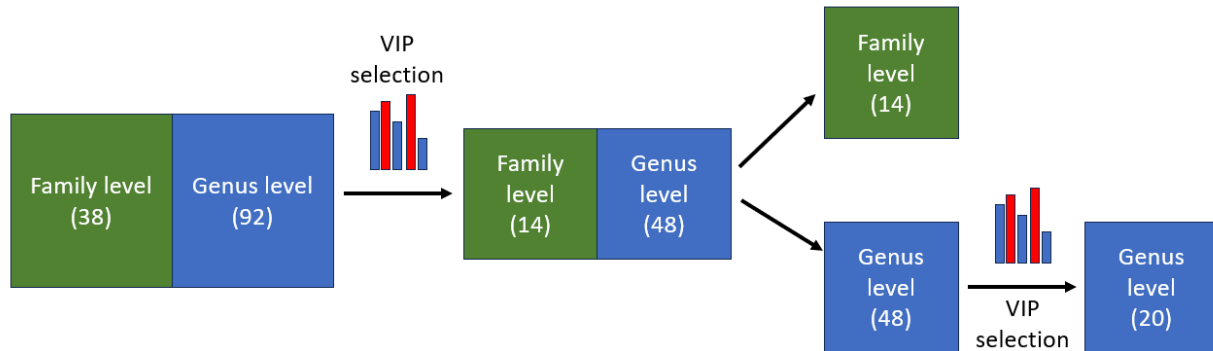
**Hypothesis:** Post-surgery, bacterial composition will change, and biomarker levels will reflect improved metabolism, resulting in an overall enhancement in patients' quality of life compared to pre-surgery conditions.

## Materials and Methods

The dataset includes 140 bacterial variables (38 at the family level and 92 at the genus level), seven clinical biomarkers, two weight measurements, and a quality of life survey (Moore survey) for seven patients, collected before surgery and six years post-surgery. The biomarkers analyzed are ghrelin (GHR), glucose (GLU), hemoglobin (HbA1c), cholesterol (CHO), interleukin 6 (IL6), high-density lipoprotein (HDL), and low-density lipoprotein (LDL).

Data analysis was done with python 3.12 and R 4.4.1. Preliminary study was done by using Grubbs test to detect outliers, cluster analysis to investigate sample similarities and Partial Least Squares Discriminant Analysis (PLS-DA) to study classification of two dataset.

The main analysis focused on bacterial and biomarker data to explore their relationships. An iterative data selection process was conducted using the VIP (Variable Importance in Projection) plot, as outlined in the process flowchart (Figure 1). PLS (Partial Least Squares) and coefficient plots were then utilized to investigate associations among bacterial families, biomarkers, and the effects of surgery. Differences between pre- and post-surgery conditions were assessed using ANOVA or the Kruskal-Wallis test, with results visualized through bar charts or box plots.



**Figure 1.** Flowchart of microbiome data selection process for analysis.

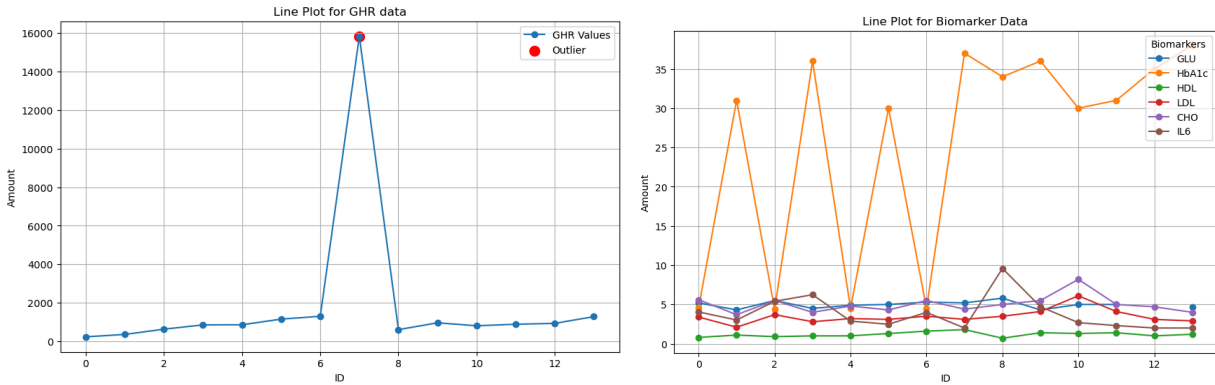
## Results

### Preliminary study

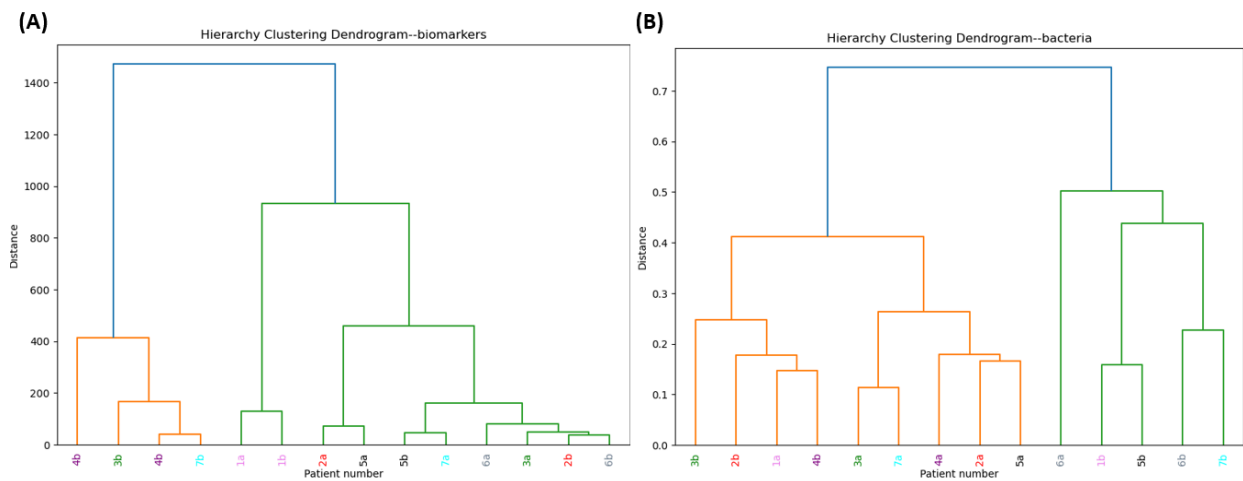
By using Grubbs test, in total 647 outliers were found in the microbiome data. When visualized in a line plot (Figure A.1, Appendix), it can be seen that the differences are not obvious, with most variation simply reflecting differences before and after surgery measurements. So to ensure data authenticity, no changes were made. As for the biomarkers, 17 outliers were detected, however, most values fluctuate within a similar range across patients (Figure 2), likely due to individual variations. An exception was a ghrelin level exceeding 15,000, which appeared to be a misrecord of the data, so it's replaced by its possible correct value. Additionally, a missing value in the glucose column was found, which was replaced by the mean value of other Y6 patients. A summary of these adjustments is presented in Table 1.

**Table 1.** Issue with data and modification.

	Issue of column	Adjustment
GHR	Outlier: 15930	1593
GLU	Missing data	5.3



**Figure 2.** Line plot of biomarker data, with the outlier in GHR data highlighted (left).



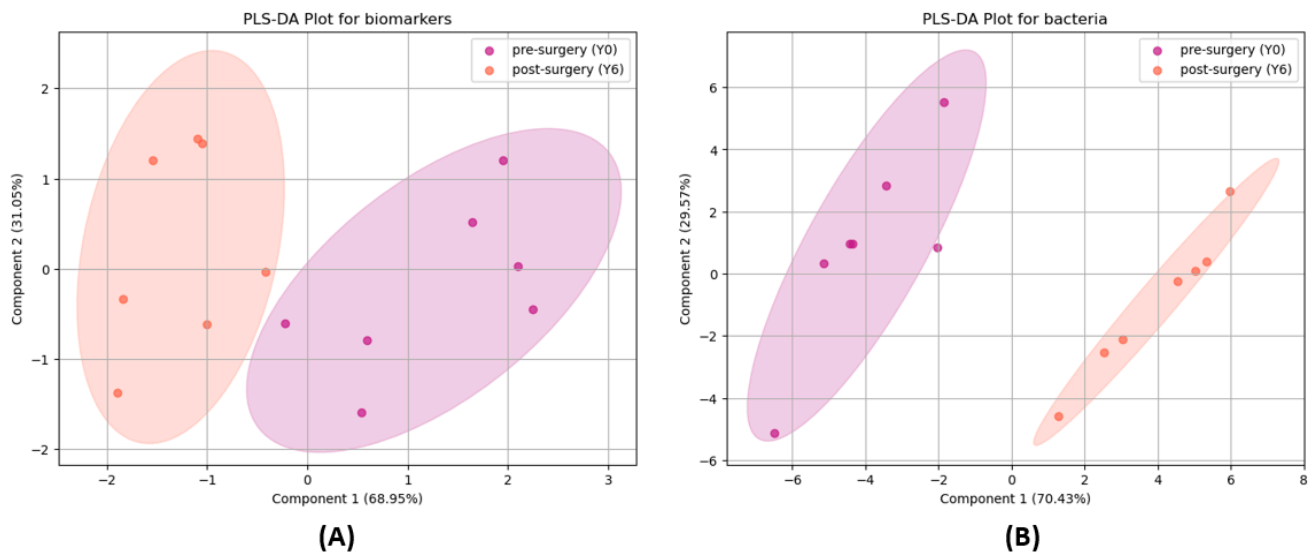
**Figure 3.** Cluster analysis of patient biomarkers (A) and bacterial composition differences before (a) and after surgery (b).

The result from cluster analysis shows if the surgery significantly altered the patients profile. In Figure 3 (A), it can be seen that patients 1, 4, 5 and 6 have close clusters between treatments (a and b), indicating the surgery did not lead to substantial changes in their biomarkers and weight or even life quality, as these samples are quite similar even after the surgical intervention. But patients 2, 3 and 7 have large differences between pre- and post-surgery. In Figure 3 (B), it can be observed that most of the patients' bacterial compositions have experienced changes after the surgery, within which patients 1 and 7 have largest changes.

The normality of data (biomarkers, physical measurement and survey) were tested by using the Shapiro-Wilks test, which shows that except HbA1c, LDL and CHO, all others are normally distributed. Normality testing for biomarkers was conducted only on selected bacteria, with results presented in a later section.

PLS-DA analysis revealed significant differences in microbial communities between pre- and post-surgery samples (Figure 4). This suggests that bariatric surgery alters both biomarkers and microbiota composition. In plot A (biomarkers), component 1 explains 68.95% of the variance while component 2 explains 31.05%. In Plot B (bacteria), component 1 explains 70.43% of the

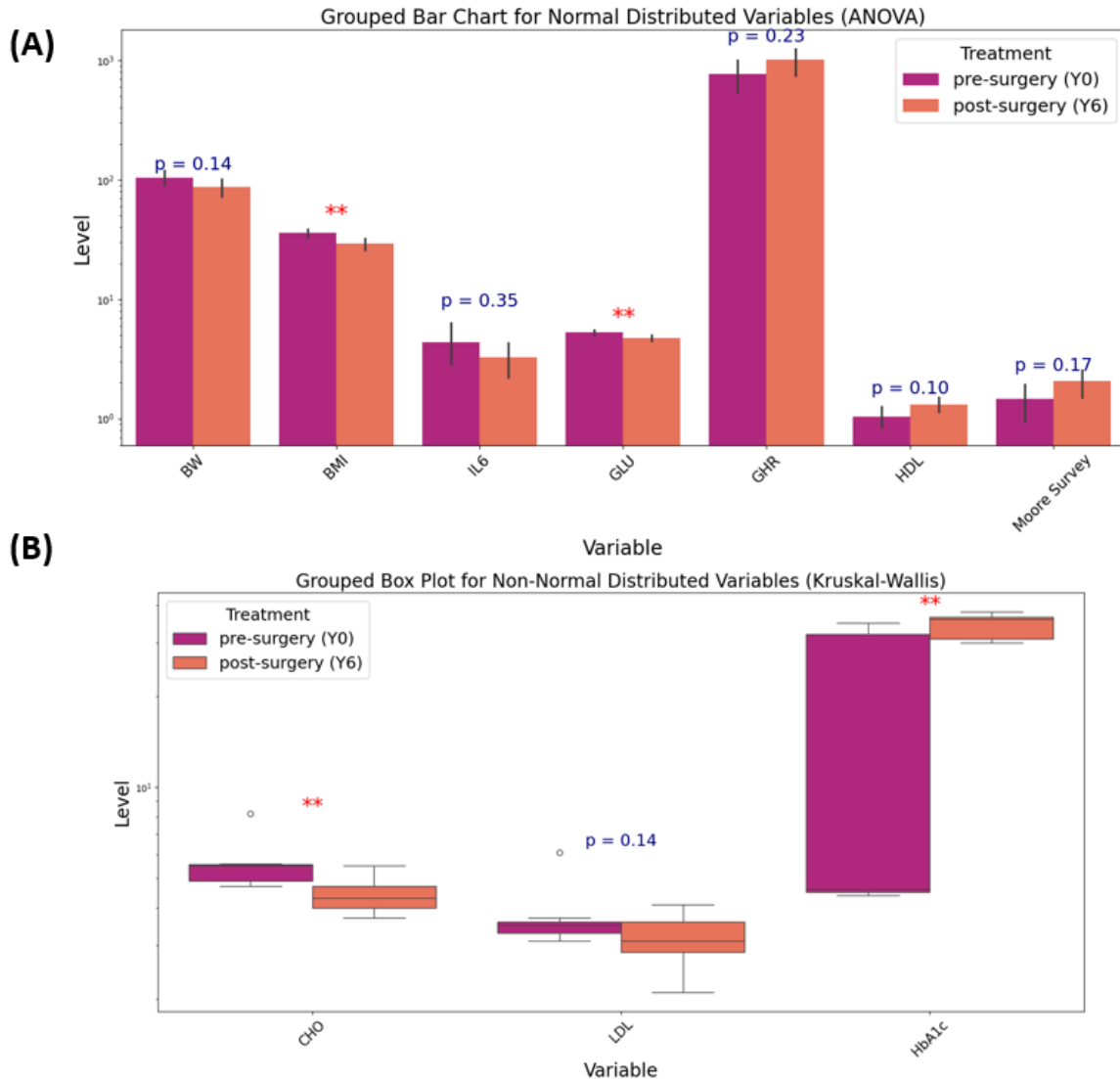
variance, and component 2 explains 29.57%. Hence it can be concluded that these two models both classify the treatment well with strong variance explained by two components. However, the confidence ellipses in plot A almost have slight overlap between the pre-surgery and post-surgery groups while in plot B doesn't, indicating a less distinct classification of biomarkers than bacteria.



**Figure 4.** Partial Least Squares Discriminant Analysis (PLS-DA) plot of biomarkers (A) and microbiome (B) before and after surgery.  $P = 0.001$  and  $0.002$  were obtained by PERMANOVA respectively.

## Biomarkers, body weight and life quality study

Figure 5 displays the results of ANOVA and Kruskal-Wallis tests, illustrating the significance of differences in biomarkers and survey scores between pre- and post-surgery samples. Six years after operation, patients show improvements in BW, BMI, all biomarkers, and overall quality of life. However, only the changes in BMI, GLU, CHO, and HbA1c were statistically significant. A detailed summary of these results is provided in Table A.1 in the Appendix. These findings suggest that surgery not only aids in weight reduction but also significantly decreases insulin resistance—a common issue among obese patients, and lowers the risk of heart disease.



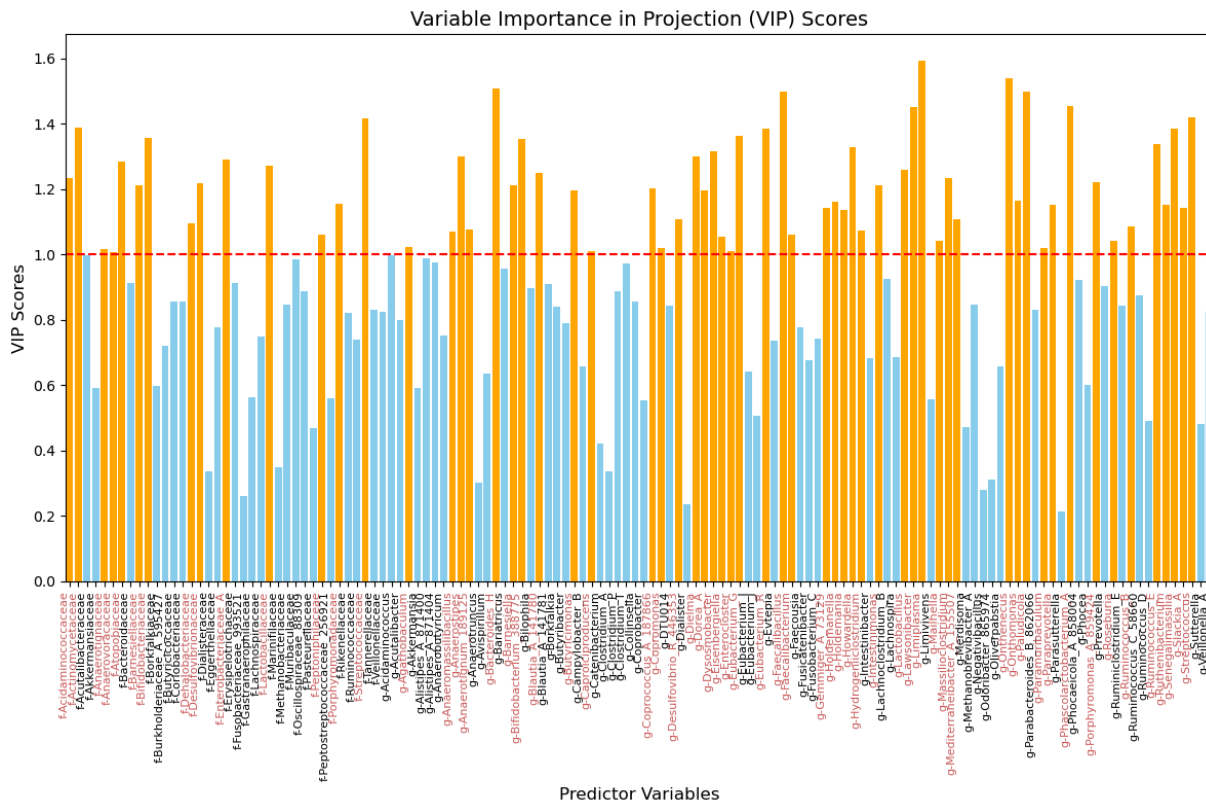
**Figure 5.** Bar chart (for normal distributed data) and box plot (for non-normal distributed data) showing differences in variables before and after surgery.

## Microbiome study

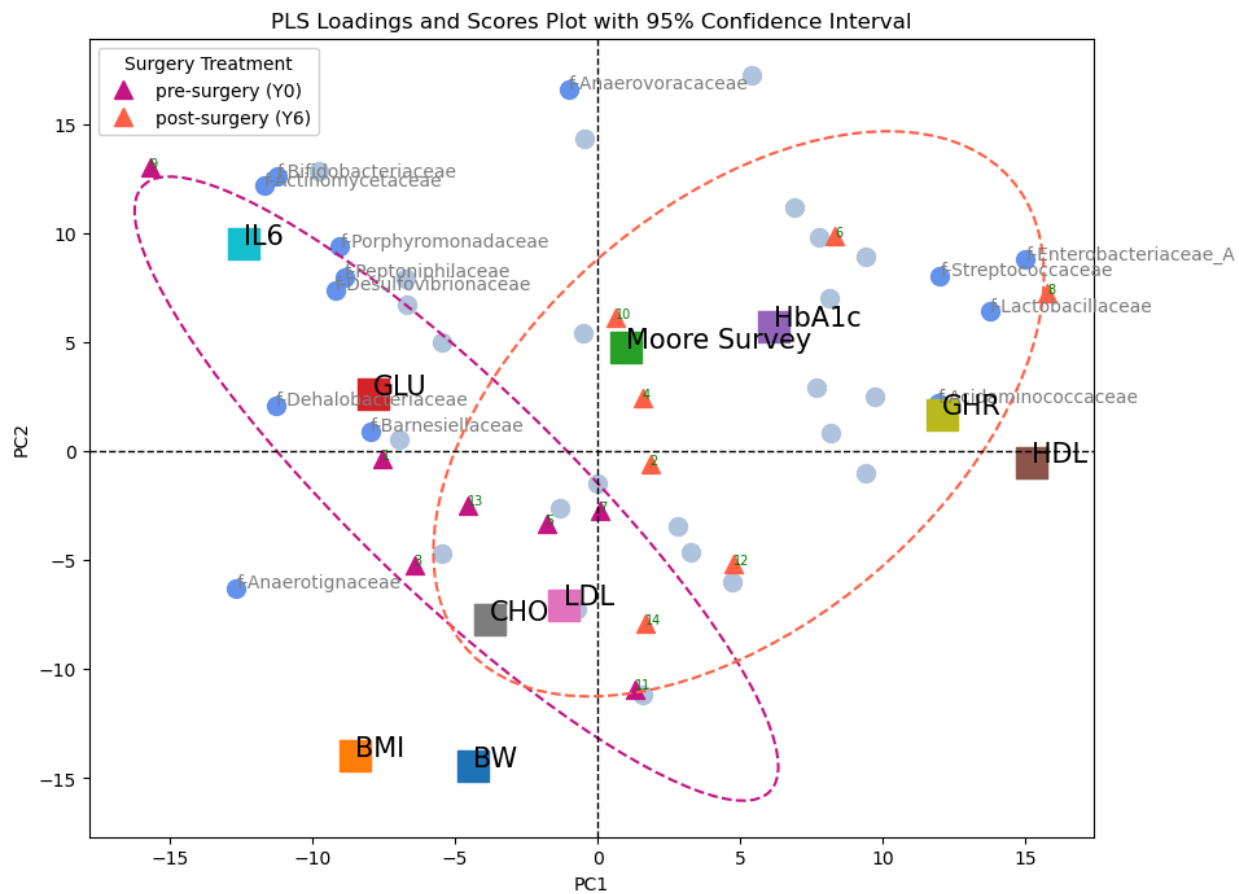
A VIP plot (Figure 6) was first generated to identify bacteria contributing significantly to biomarkers, from which 62 bacteria (13 at the family level and 49 at the genus level) out of 132 were selected. This was followed by a PLS plot (Figure 7) to examine relationships among bacterial families, biomarkers, and treatment. The analysis shows that biomarkers LDL, GLU, CHO, and IL6 are associated with pre-surgery samples, while HbA1c, HDL, and GHR align more closely with post-surgery samples. As expected, BMI and BW are higher in pre-surgery samples, while Moore Survey scores are higher post-surgery.

Among the biomarkers, LDL, CHO, GLU, and IL6 positively correlate with each other, while showing a negative correlation with HbA1c, HDL, and GHR. Additionally, it can be seen that

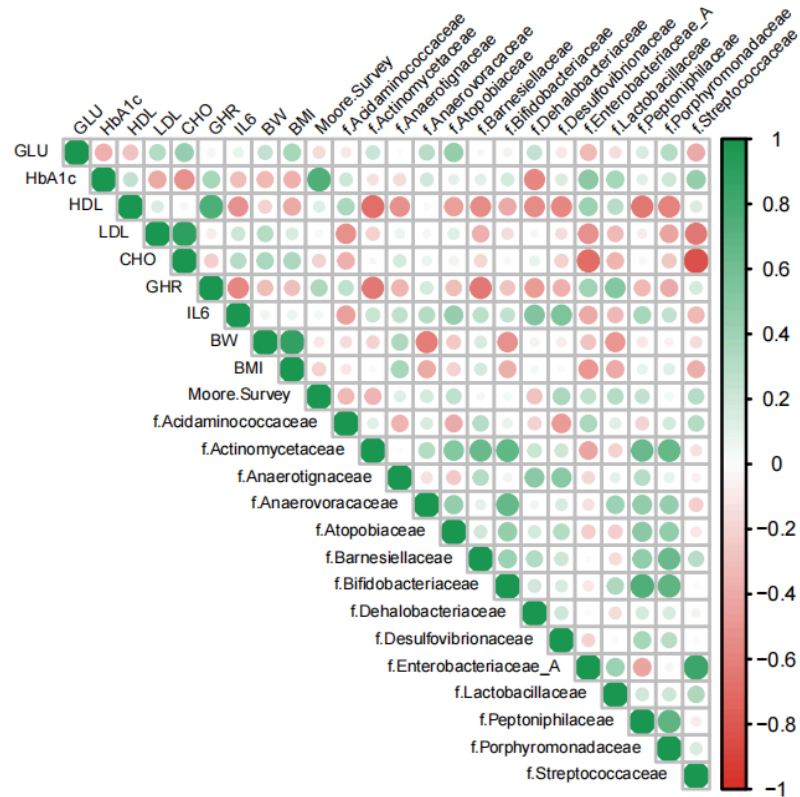
these bacterial families have relationships with biomarkers; for instance, *f-Dehalobacteriaceae* and *f-Desulfovibrionaceae* exhibit strong positive correlations with IL6, while *f-Barnesiellaceae*, *f-Desulfovibrionaceae*, and *f-Peptoniphilaceae* have strong negative correlations with HDL.



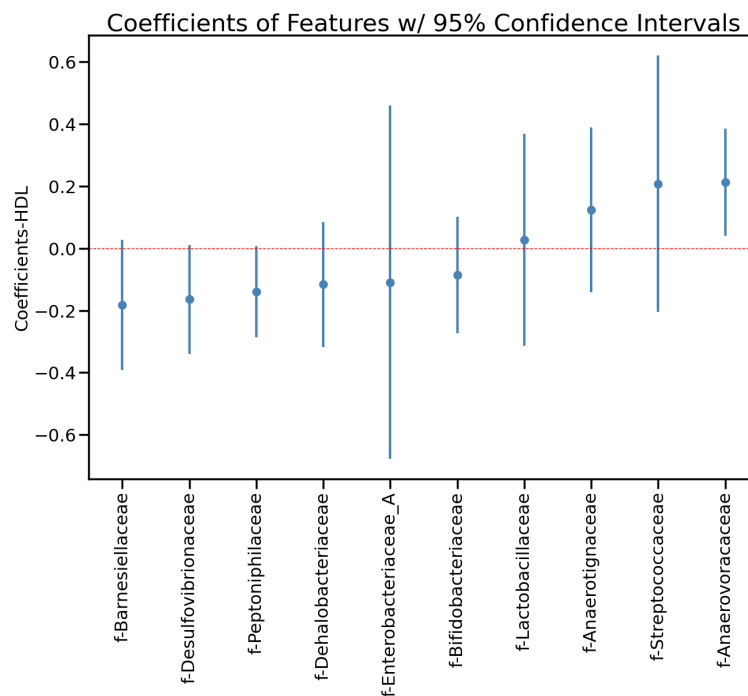
**Figure 6.** VIP plot with important bacteria (scores >1) highlighted in orange.



**Figure 7.** PLS plot with important family level bacteria highlighted in darkerblue.



**Figure 8.** Spearman correlation analysis plot with relationship among family level bacteria, biomarkers and survey data shown.



**Figure 9.** Example of coefficient plot with HDL as coefficient and selected bacteria as explanatory variables.



To further investigate the changes in gut bacteria after surgery, genus level bacteria were added into PLS to find new insight (Figure 10). A subsequent VIP plot (Figure 10) identified 20 out of 48 genus-level bacteria as significant contributors to the biomarkers. Of these, 11 were selected for coefficient plotting to study their relationships with biomarkers. A summary of these results, along with the previously analyzed family-level bacteria, is provided in Table 2.

The results indicate that *g-Porphyromonas\_A\_859424*, *g-Dielma*, *g-Bacteroides\_H*, *g-Bifidobacterium\_388775*, and *g-Anaerostipes* have significant impacts on biomarkers. GHR shows a strong positive correlation with *g-Porphyromonas\_A\_859424* and *g-Bacteroides\_H*, but has negative correlation with *g-Dielma* and *g-Anaerostipes*. Similarly, HbA1c has a strong positive correlation with *g-Porphyromonas\_A\_859424*, while showing a negative correlation with *g-Bifidobacterium\_388775* and *g-Anaerostipes*. IL6 is positively correlated with *g-Bacteroides\_H* and negatively correlated with *g-Dielma*. However, these findings are not entirely consistent with those from the correlation plot (Figure 12), the possible reasons are discussed in detail in the discussion section.

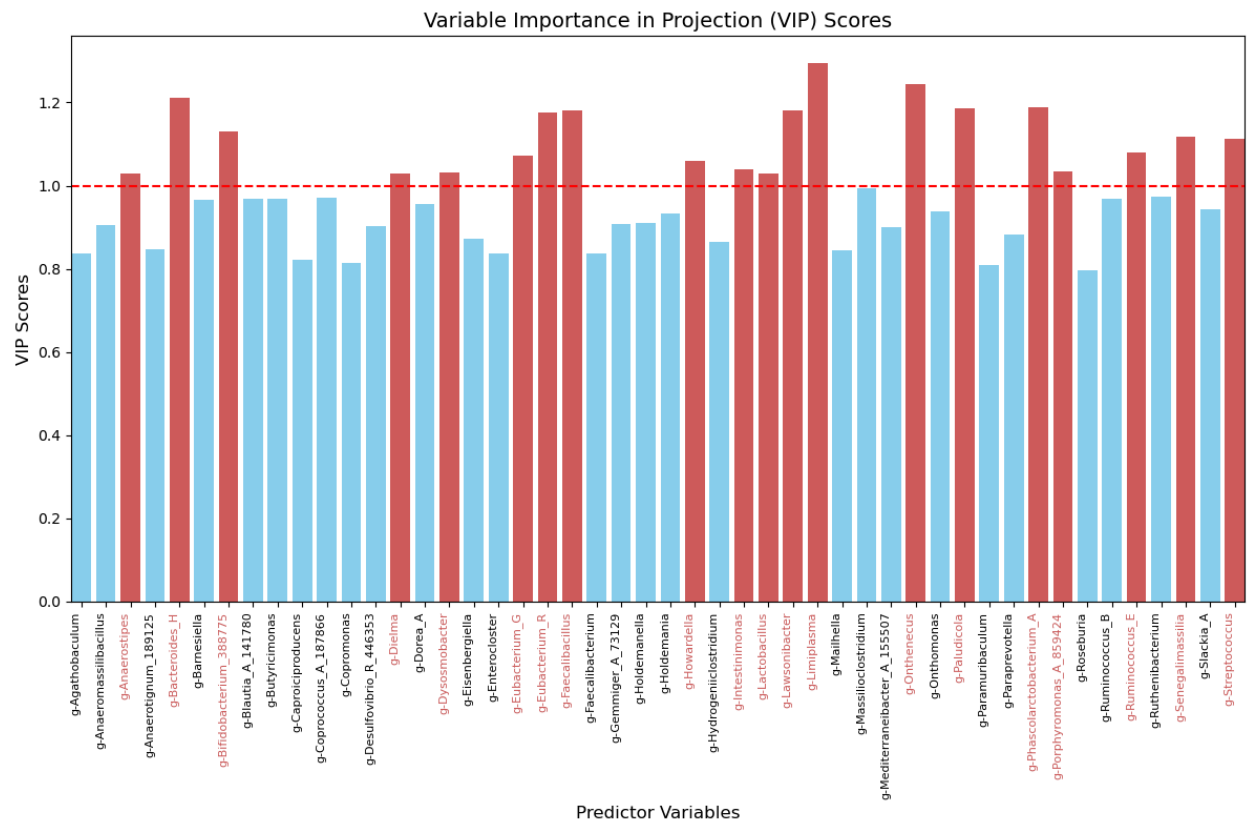
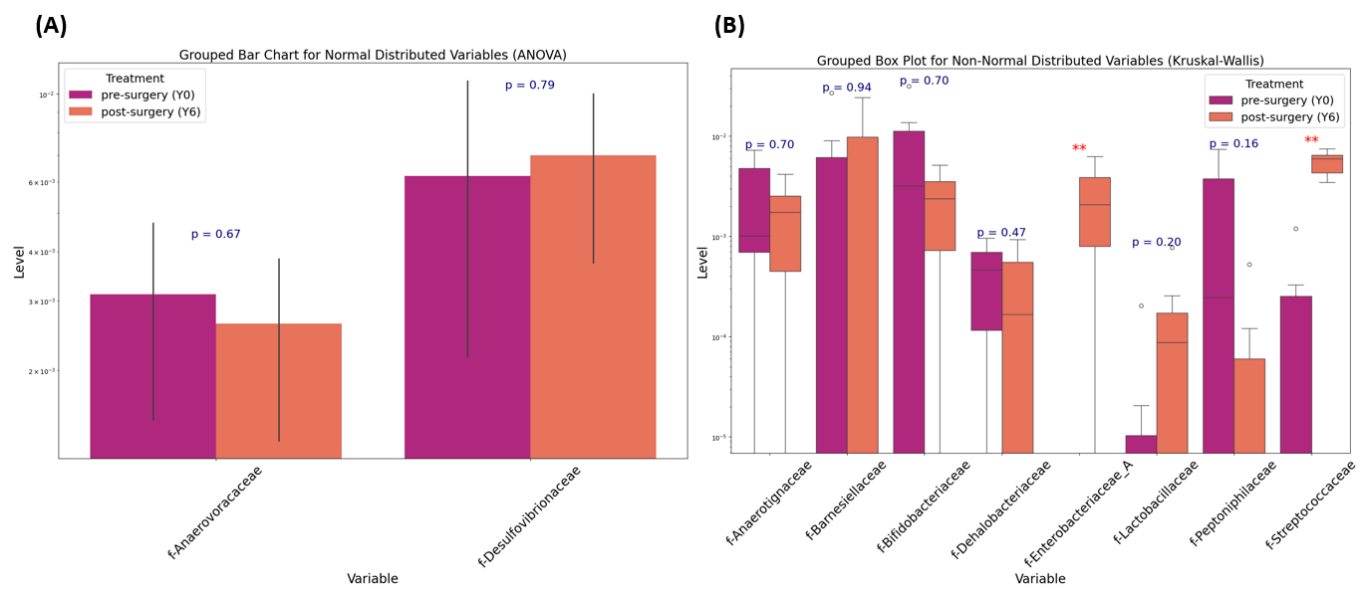


Figure 10. VIP plot for selecting important bacteria within genus level

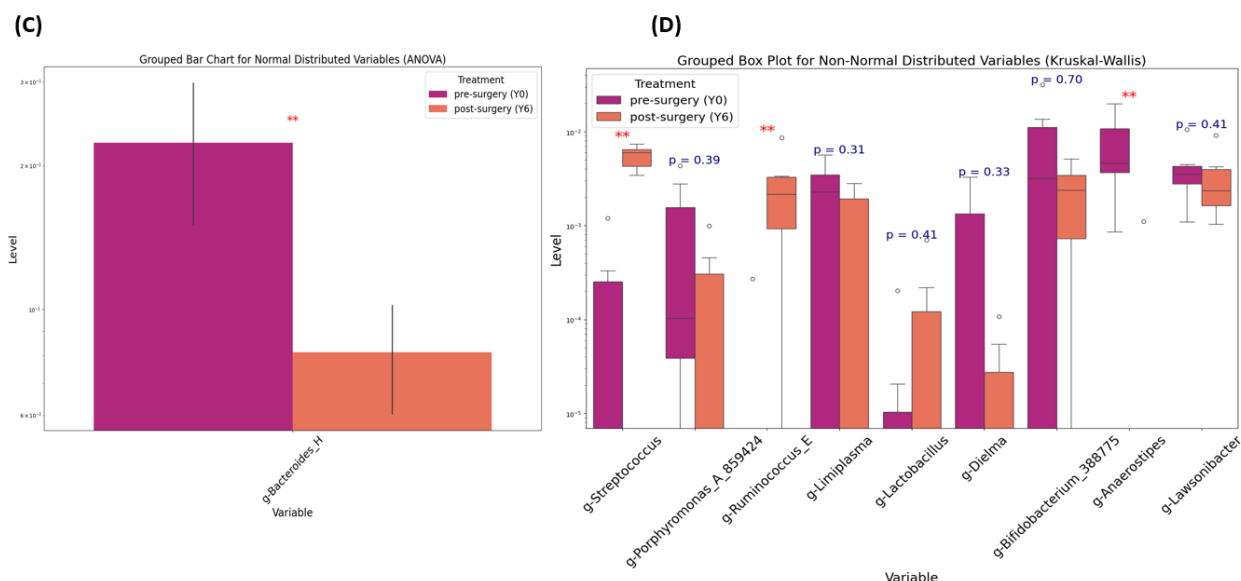




ANOVA and Kruskal-Wallis (KKW) tests, along with bar charts and box plots, were used to analyze changes in all genus- and family-level bacteria identified as important variables, comparing pre- and post-surgery conditions. The results, displayed in Figure 13 (family) and Figure 14 (genus), indicate that most bacteria experienced change after surgery. It's notable that *f-Enterobacteriaceae\_A*, *f-Streptococcaceae*, *g-Bacteroides\_H*, *g-Ruminococcus\_E*, and *g-Anaerostipes* showed significant changes. A detailed summary of these results is provided in Table A.2 and Table A.3 in Appendix, supporting the conclusion that surgery significantly alters bacterial composition, which may reflect improvements in gut microbial community structure and metabolism. Additionally, when looking at the phylum level, it can be found that the *Firmicutes/Bacteroidota* ratio and the abundance of *Proteobacteria* increased after surgery.



**Figure 13.** Bar chart (for normal distributed data) and box plot (for non-normal distributed data) showing differences in family level bacteria before and after surgery.



**Figure 14.** Bar chart (for normal distributed data) and box plot (for non-normal distributed data) showing differences in genus level bacteria before and after surgery.

## Discussion

This research confirms changes in the gut microbial community and health biomarkers, along with their relationships, in patients who underwent bariatric surgery. Six years post-surgery, patients continue to show reduced weight, glucose levels, and cholesterol, suggesting decreased insulin resistance (a factor in high blood glucose levels) and fewer fatty deposits in blood vessels.

An improved Firmicutes/Bacteroidota ratio, associated with reduced obesity and better metabolic health, was observed, aligning with findings from previous studies on Chinese patients after Laparoscopic Sleeve Gastrectomy [3][4]. Additionally, bacteria like *Lactobacillus*, *Bifidobacterium*, *Prevotella*, and *Clostridium* have shown correlations with leptin and ghrelin levels [5], while *Lachnospiraceae* and *Ruminococcus* are positively linked to lipid metabolism markers like LDL-C and HDL-C—though we did not observe this in our data. Furthermore, the increase in *Proteobacteria* is consistent with findings by Mohammadzadeh et al. [6]. Future research could benefit from a closer examination of relative abundance changes in bacterial taxa if additional data becomes available.

There are several limitations in this study. Firstly, some studies suggest that certain bacteria, such as *Lactobacillus*, show notable reductions following Roux-en-Y Gastric Bypass (RYGB) but not with Sleeve Gastrectomy (SG), indicating that bacterial changes may differ based on surgery type. However, because the dataset does not specify the type of surgery performed, we cannot compare outcomes by surgery type. Additionally, studies have shown that some bacteria

level (such as *Ruminococcaceae*) tend to increase with age, with elderly and long-lived individuals (> 90 years old) having significantly higher levels than younger populations. Given the age differences among the patients in this study, this could account for some of the observed variations in bacteria, potentially leading to an increased risk of type I error in statistical analysis. Furthermore, the sample size of only seven patients is quite limited; a larger sample set would likely provide a more robust characterization of biomarkers and bacteria changes. Due to this reason, building a model for training, testing and classification becomes tricky.

It's suggested that coefficient plot might not be the best technique in analyzing data that has a more variance than samples (more columns than rows) due to the multicollinearity of the variables affects the standard errors of the coefficients, hence changing estimated values and statistical significance. Also, if more explanatory variables are selected than sample number, the model will experience overfitting and lead to error in calculating standard errors. In this case, the result from Spearman correlation analysis might be more meaningful.

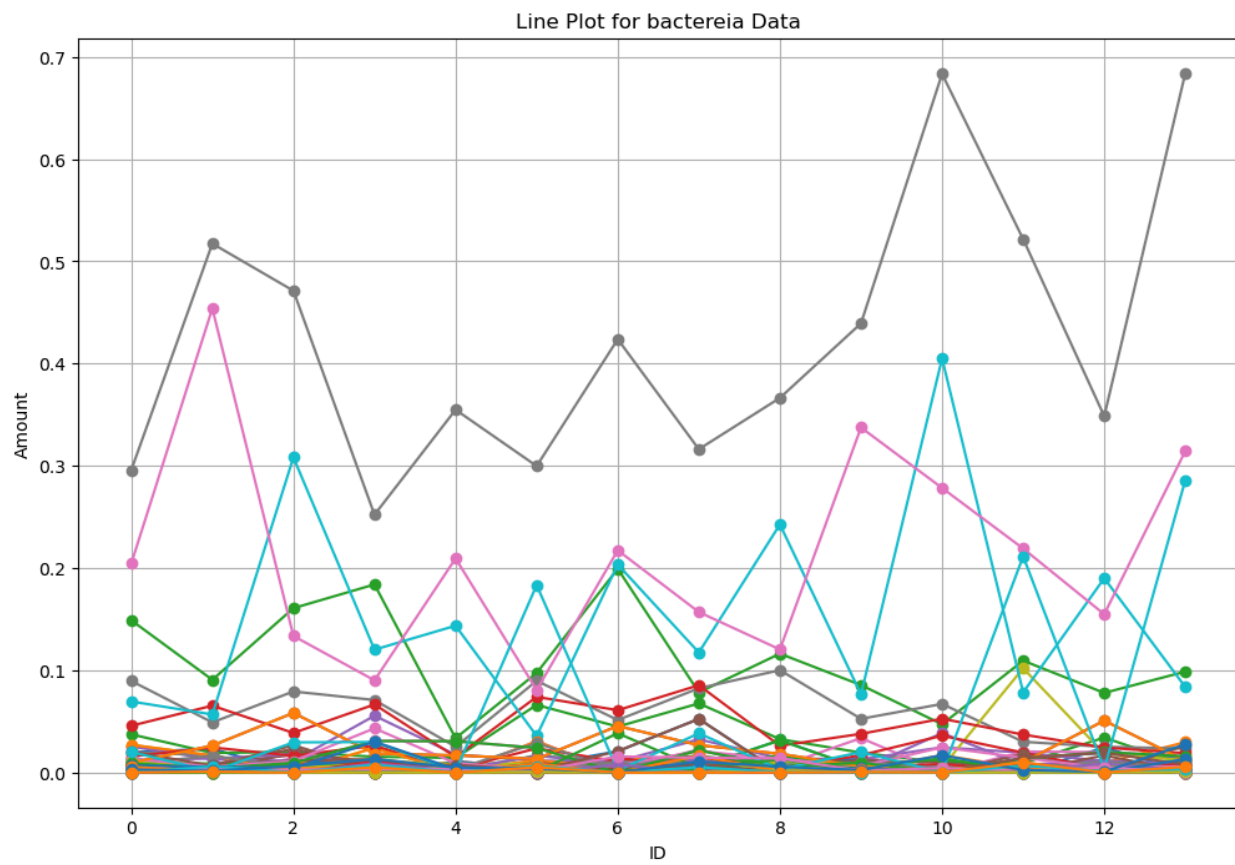
Further research can be conducted on using external microbiome analyzing tools for getting more diverse information. Moreover, it would be interesting to track diet change after surgery that contributes to microbiome composition change. Last but not the least, researching the difference between different surgery types.

## Conclusion

In conclusion, this study illustrated that bariatric surgery improves both microbiota composition and clinical biomarkers, leading to enhancements in overall health and life quality. Six years after surgery, significant reductions in BMI, glucose, and cholesterol levels were found. The Firmicutes/Bacteroidota ratio and Proteobacteria increased, supporting previous findings on post-surgical microbiome changes.

Despite the study's promising findings, limitations such as small sample size, potential surgery-type effects, and age-related bacterial variation cause error in the study and hence need further research.

## Appendix



**Figure A.1.** Line plot for bacteria data.

**Table A.1** Pre-, post-surgery biomarkers, physical measurements and survey comparison.

Variable	Pre-surgery	Post-surgery
BW	104.7±7.9	86.6±8.1
BMI*	36.1±1.6	29.3±1.8
Moore survey	1.5±0.3	2.1±0.3
IL6	4.4±0.9	3.3±0.6
GLU*	5.3±0.1	4.7±0.1
CHO*	5.6±0.5	4.4±0.2
GHR	769±124	1012±145
LDL	3.8±0.4	3.2±0.3
HbA1c*	16.7±5.8	34.1±1.3
HDL	1.1±0.1	1.3±0.1

**Table A.2** Pre-, post-surgery family level bacteria comparison

Bacteria	Phylum	Pre-surgery (*10 <sup>-4</sup> )	Post-surgery (*10 <sup>-4</sup> )
f-Anaerotignaceae	Firmicutes_A	27±11	17±5.2
f-Barnesiellaceae	Bacteroidota	56±37	62±37
f-Bifidobacteriaceae	Actinobacteriota	81±43	23±7.3
f-Dehalobacteriaceae	Firmicutes_B	4.4±1.4	3.1±1.4
f-Enterobacteriaceae_A*	Proteobacteria	0.0±0.0	25±8.6
f-Lactobacillaceae	Firmicutes	0.3±0.2	1.7±1.0
f-Peptoniphilaceae	Firmicutes_A	22±12	0.9±0.7
f-Streptococcaceae*	Firmicutes	2.4±1.6	50±9.6
f-Anaerovoracaceae	Firmicutes_A	31±9.0	26±6.8
f-Desulfovibrionaceae	Desulfobacterota_I	62±23	69±16



**Table A.3** Pre-, post-surgery genus level bacteria comparison.

Bacteria	Phylum	Pre-surgery (*10 <sup>-4</sup> )	Post-surgery (*10 <sup>-4</sup> )
g-Streptococcus	Firmicutes	2.4±1.0	49±9.0
g-Porphyromonas _A_859424	Bacteroidota	11±6.0	2.2±1.1
g-Ruminococcus_E*	Firmicutes_A	0.4±0.3	27±11
g-Limiplasma	Firmicutes_A	21±8.1	9±4.3
g-Lactobacillus	Firmicutes	0.3±0.2	1±0.9
g-Dielma	Firmicutes	8.6±5.2	0.2±0.1
g-Bifidobacterium_388775	Actinobacteriota	82±4.3	22±7.3
g-Anaerostipes*	Bacteroidota	77±25	1.5±1.1
g-Lawsonibacter	Firmicutes	42±11	34±10
g-Bacteroides_H*	Bacteroidota	2231±414	812±114

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